A distinct mechanism of the ABC transporter BtuCD-F revealed by the dynamics of complex formation

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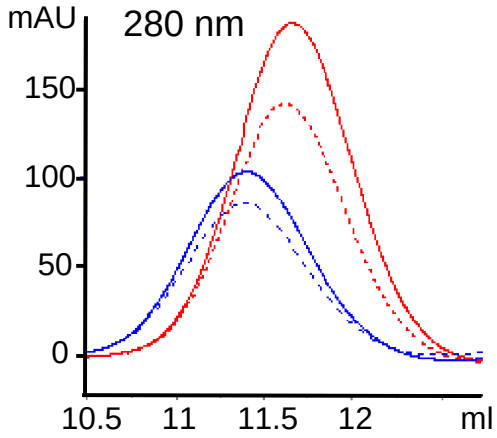
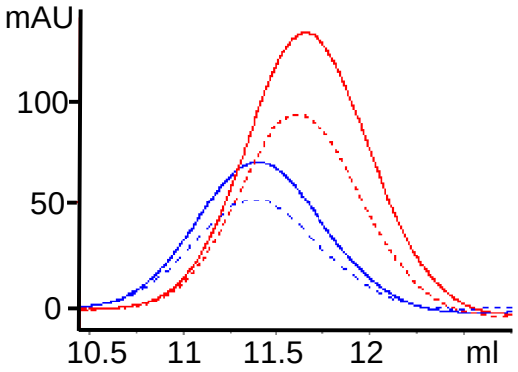
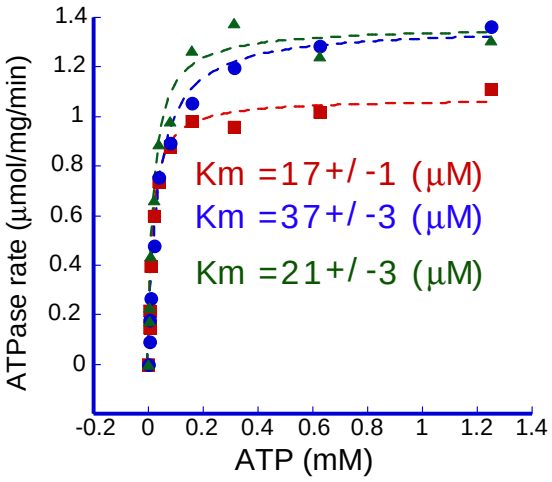
a



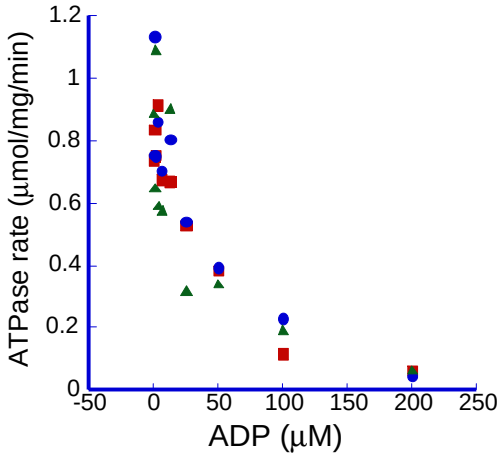


	Experimental 260/280 Ratios in the absence of ATP	Expected 260/280 Ratios with bound ATP	Experimental 260/280 Ratios in the presence of ATP/EDTA
BtuCD	0.66	0.707	0.71
BtuCD-F	0.64	0.678	0.68

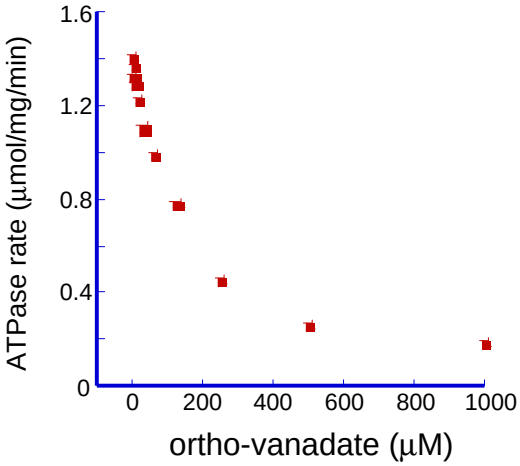
b



c



d



Supplementary Figure 1.

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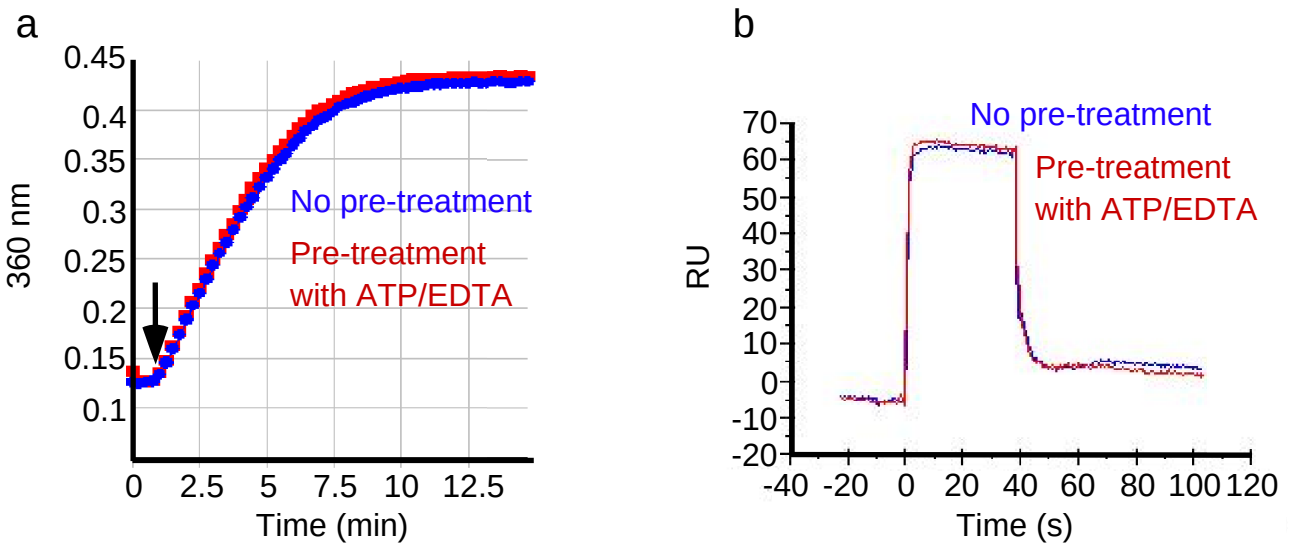
Figure 1. Interaction of BtuCD and BtuCD-F with nucleotides. (a) Binding of ATP: BtuCD (red curves) or BtuCD-F (blue curves) were subjected to size exclusion chromatography in the presence (solid lines) or absence (dashed lines) of 1mM ATP, 50 μM EDTA. Where applicable, ATP and EDTA were present in both the sample and column buffers. Absorbance was measured at 260 nm and 280 nm. The 260/280 nm absorbance ratios were calculated by integrating of the area of the protein peaks and compared to expected values obtained by adding a 2-fold molar excess of ATP to BtuCD or to BtuCD-F and measuring their 260/280 nm absorbance ratio in a spectrophotometer. (b) ATP hydrolysis: maximal ATPase rates were determined for BtuCD (red), BtuCD-F (blue), and BtuCD-F in the presence of 50 μM vitamin B_{12} (green). Apparent Km's were calculated by applying simple Michaelis-Menten kinetics. (c) Inhibition of ATP hydrolysis by ADP: maximal rates of hydrolysis of 30 μM ATP in the presence of the indicated ADP concentrations were measured for BtuCD (red), BtuCD-F (blue), and BtuCD-F in the presence of 50 μM vitamin B_{12} (green). The apparent $Ki_{(ADP)}$ for the three samples was quite similar, in the range of 25-50 μM ADP. (d) Inhibition of BtuCD ATPase activity by ortho-vanadte: maximal rates of hydrolysis of 1 mM ATP were measured in the presence of the indicated ortho-vanadte concentrations.



Supplementary Figure 2.

Figure 2. Reversibility of BtuCD inhibition by ATP/EDTA: (a) ATP hydrolysis assays: BtuCD was incubated for 10 minutes at 37C° with buffer supplemented with 50 μM EDTA, 1mM ATP (red). The ATPase activity of this sample was compared with an identical sample that was not incubated with ATP/EDTA (blue). Hydrolysis was initiated by addition of 2.5mM $MgCl_2$ (indicated by an arrow), and measured by using Molecular Probes® EnzCheck® kit, monitoring absorbance at 360 nm. (b) Biacore experiment; Two identical BtuCD samples were immobilized onto a Ni-NTA chip and washed for 5 minutes with buffer (blue) or buffer supplemented with 50 μM EDTA, 1mM ATP (red). Both samples were then washed for 5 more minutes with buffer devoid of additives. At time zero, 5μM BtuF, 300μM vitamin B_{12}, were injected to both flow-cells.

Supplementary methods

Determination of 260/280 nm absorbance ratios: Purified BtuCD or BtuCD-F were subjected to size exclusion chromatography on a Superdex200 gel filtration column in the presence or absence of 1mM ATP, 50 µM EDTA (in both sample and column buffers). Absorbance was measured at 260 nm and 280 nm. The 260/280 nm absorbance ratios were calculated by integrating of the area of the protein peaks using the AKTA™ control and analysis software Unicorn™.

ATP hydrolysis assays, inhibition of ATP hydrolysis by ADP, and inhibition of ATP hydrolysis by ortho-vanadate: ATP hydrolysis was measured using Molecular Probes® EnzCheck® kit, at 37Cº, in a 96-well format, according to the manufacturer's specifications. To initiate hydrolysis, 2.5mM $MgCl_2$ was injected to a solution containing 0.35 µM BtuCD in 25 mM Tris-HCl pH 7.5, 0.5M NaCl, 0.1% LDAO, 50 µM EDTA, and the indicated ATP concentration. Where applicable, 1.4 µM BtuF, and/or 50 µM vitamin B_{12}, or ADP, or ortho-vanadate were also present.

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